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Thesis

RELATION OF THE PITUITARY TO SKELETON FORMATION

by

Robert Lester Levine

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TABLE OF CONTENTS

I. Introduction i

II. The Anatomy of the Pituitary Gland 1

A. Gross Structure 1

B. Embryology 3

C. Microscopic Structure 6

1. Pars Anterior 6

2. Pars Intermedia 9

3. Pars Posterior 9

III. The Principles or Hormones of the Gland 11

A. Secretion of the Anterior Lobe 13

IV. Experimental Dwarfism and Gigantism 14

A. The results of partial and total hypophysectomy 14

B. The effects of replacement therapy 17

V. The Growth Hormone. 20

A. The effect of administration 20

B. Preparation and Properties 33

C. The Mechanism of Action 35

VI. Summary 38

VII. Bibliography

Figures and Tables

Figure 1 ----------Page 2 b

Table 1 ----------Page 24

Table 2 ----------Page 25

Table 3 ----------Page 28

Table 4 ----------Page 31

I INTRODUCTION

The pituitary body or hypophysis cerebri can rightfully be called the "general of the endocrine series" because of the many principles or hormones that are claimed to be present in the gland. According to Cushing (1912), the gland was recognized from the time of Galen, with no particular function attributed to it. Some investigators considered it another lymph gland, others believed it to be a nasal mucous secreting gland. This was the position held until 1887, when Minkowski interpreted Marie's description of acromegaly, to a hyperfunctioning of the pituitary gland. From this period to the present day, the literature has grown very much.

The early literature was concerned with attempts to produce acromegaly or some of the symptoms associated with it by feeding experiments, hypophysectomy partial or total. The results obtained by many of the early workers have been either discarded or have been supplemented by a great many workers. However, the contributions of Paulesco, Biedle, Cushing and Bell have been very illuminating, in the research work that followed.

This thesis is primarily concerned with the experimental work that finally brought about the fact that there is a growth stimulating hormone, which influences the body and also the skeleton. The author of this thesis attempts to present a concise review of the literature whereby the growth hormone of the anterior lobe of the pituitary has influenced

skeletal growth as well as body growth, for according to Handelsman and Gordon (1930) the increase in body weight is roughly parallel to the increase in bone growth.

The clinical investigations of the diseases of the pituitary body will not be discussed in this paper.

II The Anatomy of the Pituitary Gland

A. Gross structure

The pituitary body is built up of two unrelated parts, composed of different types of tissue, one of which is typically glandular, the other related to nervous tissue. The two parts come together early in foetal life.

The anatomy and histology of the gland follows that of Percival Bailey in Cowdry's "Special Cytology."

The human hypophysis is more or less a spherical organ lying beneath the brain in the sella turcica of the sphenoid bone. It is covered by an extension of the dura mater known as the diaphragma sellae, which is pierced by a narrow stalk uniting the organ with the brain. The dura mater also lines the sella so that it comes thus to form a continuous capsule within which the subarachnoid space extends everywhere except at the extreme posterior extremity where the blood vessels enter the posterior lobe. Cushing (1930) says, "No other single structure in the body is so doubly protected, so centrally placed, so well hidden."

The size and weight varies, but the average weight is about .57 grams. It is ovoid in shape. Rasmussen gives its average dimensions as 10 mm antero-posteriorly, .6 mm dorso-ventrally and 13 mm from side to side.

It is customary to recognize two lobes, an anterior and a posterior, separated by an epithelial lined cleft. This division however is merely gross. The posterior lobe when examined under a microscope is seen to be composed of an inner

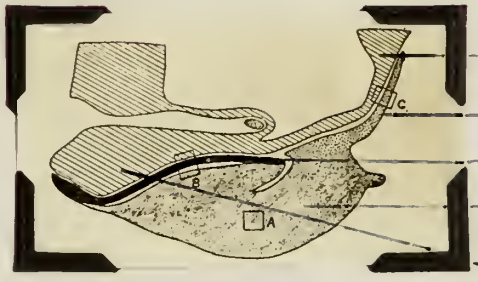
core, the pars nervosa, which is an extension from the hypothalamic region of the brain, while an outer epithelial covering, the pars intermedia, is composed of cells which are continuous at the posterior extremity with similar cells of the anterior lobe.

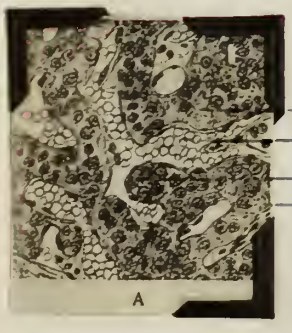
The anterior lobe or pars distalis is more homogeneous. From it a thin layer of cells, the pars tuberalis, spreads out over the base of the brain.

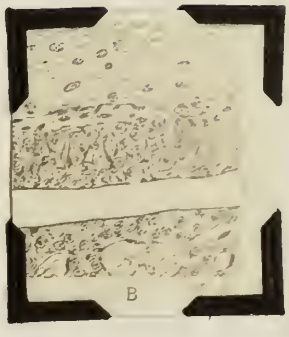
The various portions can be distinguished in every animal, but vary in their size and relationships, the similarity being much more parallel in the foetal stages of development. Engelbach (1932) has summarized the divergences in different animals: "The three mammalian types of hypophyses are exemplified in the cat, the dog, and man. In the cat, the posterior lobe is hollow and its cavity is in free communication with the third ventricle of the brain. The epithelium of the anterior lobe almost completely surrounds the posterior lobe. In the dog, the body of the posterior lobe is solid, but the neck is hollow and communicates with the third ventricle. As in the first type, the posterior lobe is almost completely surrounded by epithelium. In man (monkey, ox, pig and rabbit), the body and neck of the posterior lobe are solid, although traces of a cavity are occasionally found in the neck. The epithelium of the anterior lobe does not spread so far around the neck and spreads over and into the adjacent surface of the brain (Herring)."

The epithelial lined cleft between the anterior and

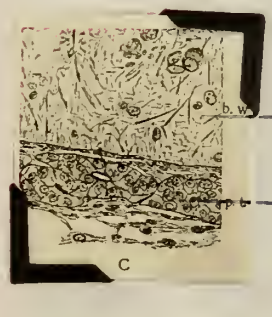
Figure 1







N.L.= Pars Nervosa
P.Int= Pars Intermedia
Ant.L.= Anterior Lobe



After Atwell

posterior lobes is present in the hypophyses of all mammals except adult man, where it is either completely obliterated or persists as isolated cystic cavities which may be distinguished from other cavities in this region, because they are lined with ciliated epithelium.

B. Development of the hypophysis.

Vincent (1925) wrote that Rathke in 1838 was the first to point out the double origin of the hypophysis. He believed that it occurred as an epithelial invagination at the upper part of the buccal cavity. This invagination was thought by Rathke to be of entodermal derivation. Other observers of this period looked upon the hypophysis as part of the brain. Luschka called the body a "nerve gland," consisting of two parts, separated by pia mater. Ecker includes the structure of both portions under the name of "Blutgefassdrusen. F. M. Balfour in 1874 showed that it really comes from the buccal ectoderm (elasmo-branch fishes), and this was confirmed by Gotte in 1875 for Amphibia.

Mihalkovics (1875) greatly elucidated the early development of the pituitary body in the rabbit and the chick. According to his work, the anterior lobe is developed from Rathke's pouch and is ectodermal. The beginning is in front of the oral plate. When this ruptures, its upper stump, containing in its upper part the head of the notochord, bends forward and narrows the mouth of the epithelial pouch, leading to the formation of a definite sac--the hypophysial sac. The wall of the sac presses upon the base of the anterior brain

vesicle, giving rise, at its upper extremity, to a fold in the wall of the brain, which becomes the primitive infundibulum. The primitive infundibular process comprises the surrounding tissue of the tuber cinereum as well as the origin of the infundibulum. The true infundibulum is formed at a later stage by its own growth from a portion of the primitive infundibular process. The head of the notochord acts as a barrier to the backward growth of the sac, but takes no part in the actual formation of the hypophysis.

Mihalkovics' work has been confirmed in the main by most writers. Kupfer (1894) described an additional origin of part of the anterior lobe from the entoderm of the foregut, and this view has also received some support. Herring (1908) however believes that the epithelial portion of the pituitary is entirely ectodermic. His work briefly is as follows:

The pituitary begins to develop very early in the embryonic life of the animal. The epithelial portion is derived completely from the ectodermic wall of "Rathke's Pouch." Its origin is single and mesial. The epithelium is distinguished early into two parts, the intermediate closely adheres to the wall of the cerebral vesicle; the cells are clear and tend to form a colloid. In further development of the glandular portion of the organ from the epithelium which forms the walls of the enlargement of 'Rathke's Pocket,' there is a great multiplication of cells of the anterior wall which form trabeculae subdivided later by connective tissue and blood vessels in isolated clumps. The mass thereby produced becomes

the anterior of the gland. The posterior wall however is not so thick, but becomes firmly adherent to and eventually in continuity with the pars nervosa. The remainder of the invagination cavity becomes the intraglandular cleft. The pars tuberalis is developed later from two lateral diverticula of the main hypophyseal vesicle. They ultimately fuse together in the mid line around the stalk and spread over the under surface of the tuber cinereum. The cavities become obliterated and the cells of their walls form the substance of the pars tuberalis; they are often collected in vesicles, which contain a colloid material.

The pars nervosa is developed from the hollow process of neural ectoderm which is in contact with the posterior wall of the buccal invagination: with which, as stated above, it eventually comes into complete continuity. In man and in most mammals the cavity of the hollow process becomes obliterated, but in some (e.g. the cat) it persists throughout life.

P. E. Smith (1920) found that if the buccal in growth in young tadpoles (4 mm) is removed, the infundibulum is not developed and the floor of the third ventricle remains membranous. If only a small piece of the buccal ingrowth epithelium is left behind, the part of the neural ectoderm with which it is left in contact becomes thickened and undergoes further development.

The relationship of the various parts of the hypophysis may be represented in the following schema after Bailey:

Pars tuberalis

Pars buccalis Pars distalis----Anterior lobe.

Pars intermedia

Posterior lobe.

Pars nervosa

Vascular, Lymphatic and Nervous Supply.

The pars tuberalis and pars distalis have a very rich blood supply from numerous small blood vessels which arise from the circle of Willis and descend in the pia mater of the infundibulum along the stalk. The pars nervosa has a smaller blood supply which enters from its postero-inferior extremity where it is not invested by the pars buccalis. The pars intermedia is very poorly vascularized in all mammals, although numerous small vessels run in the connective tissue representing the pia mater which separates it from the pars nervosa.

The pars distalis receives its nerves from the carotid plexus. Fine unmyelinated nerve fibers follow the vascular supply. Branches leave at intervals to cross over the cellular columns and end between the glandular cells. An important tract of fibers originates in the nucleus supra-opticus, and descends the anterior wall of the infundibulum to spread out in the nervous portion, ending in tangled masses around the blood vessels and among the cells.

Lymphatic vessels have never been demonstrated in the hypophysis.

C. Microscopic Structure.

1. Pars distalis:-

This is the best known portion of the pituitary and probably the most important. It is composed of columns of cells separated by a large number of vascular sinuses and a small amount of connective tissue.

Two main groups of cells may be distinguished, the chromophobe and chromophile, according to the intensity of their staining reactions. The chromophile cells are deeper staining and owe this to the presence of granules which are imbedded in their cytoplasm. The granules are of two types, the basophile stained only with basic dyes and the acidophiles with acid dyes. This however is not correct and they are referred to now as beta and alpha granules. The majority of investigators believe that the two types of granules are never found in the same cell.

According to Rasmussen (1929), the three types of cells in the anterior pituitary of man are:

	Extreme Value	Mean Value
	per cent	
Chromophile		
Acidophile (alpha)	23---------59	37
Basophile (beta)	4.5--------27	11
Chromophobe (Neutrophile)	32---------66	52

He finds a very similar distribution in the pituitaries of non-pregnant women (averaging 43% alpha, 7 beta and 50 chromophobe) while pregnancy causes no significant change.

The alpha granules are large, spherical and very distinct, and usually packed so close that they sometimes obscure all other structural details. They appear in the human in the third foetal month. The beta granules appear a little later,

they are finer and less distinct than the alpha granules. The chromophobe cells contain very little cytoplasm and are known as chief cells or reserve cells. Cushing (1930) states, "These tinctorially distinguishable cells are distributed somewhat indiscriminately throughout the gland, and cytologists have been at a loss to know whether they merely represent differing stages of activity of one and the same cell, or whether they have gained morphological and functional independence. It is safe to say that they have."

Biedle suggested that the chromophobe cells are "mother cells" from which the acidophile and basophile cells are derived. Severinghaus (1933) showed that the chromophobe cells of the rat pituitary can be separated into two distinct types because of their differences in the Golgi apparatus of each. The first type corresponds to the Golgi apparatus of the acidophile cells, namely a filamentous net, while that of the second corresponds to the Golgi apparatus of the basophile cells i.e. a ring. He found no evidence of a change from the alpha to the beta type, but he showed, from the study of castrated rat pituitaries that chromophile cells can revert to chromophobe cells. Small globules of lipoidal substances are seen in all the cells, but especially in the beta cells.

The nuclei of both the chromophobe and chromophile cells are of two types: some are vesicular with scattered granules of chromatin, while others are smaller with a very heavy network of chromatin. The significance of this difference is not known.

2. Pars Intermedia.

The pars intermedia is almost avascular in all mammals. The cells are of two types mainly

1. Elongated slender cells stretching the entire width of the part, which may be impregnated with Golgi method, and

2. Polygonal cells resembling the chromophobe cells of the pars distalis. These cells are generally considered to contain no characteristic granulation.

A striking characteristic of the pars intermedia of many hypophyses is the presence of a great number of cysts of varying sizes. These do not constitute regular vesicles such as are found in the thyroid. According to deBeer (1926), they are irregular cavities of vaying sizes among the cells filled with colloid and hyaline material and often with degeneration. These cavities by distention may become surrounded by a pseudo-epithelium.

Pars tuberalis.

The tuberous portion represents an insignificant part of the human hypophysis, but is fairly well developed in the dog and the cat. The cells contain no granules and often are transformed into irregular cavities in the cell columns, thus giving them a tubular appearance. The epithelial cells are scattered about on the anterior wall of the stalk.

3. Pars Nervosa.

Three different cellular elements are seen in this portion

1. typical ependymal cells with their cell bodies on the ventricular surface and processes stretching to the pial surface,

2. mossy neuroglial cells and

3. larger pyramidal or spindle shaped cells. The latter are called "pituicytes." The three cellular types may be identified by Golgi's method in the pars nervosa of the dog and cat.

Bucy in 1930 made a thorough study of the pars nervosa of the ox. He found that it was composed of nerve fibers, connective tissue and the spindle shaped cells which he called "pituicytes". The "pituicytes" are irregular shaped cells with vascular processes which end in on the blood vessels, septa of the connective tissue or the capsule. They are sometimes pigmented and are impregnated with $AgCO_3$. The connective tissue divides the pars nervosa into lobules. The nerve fibers are unmyelinated and stream down from the stalk to spread out into tangled masses in the main body of the pars nervosa. The "pituicytes" can be stained with methyl green.

III The Active Principles or Hormones in the Pituitary Body.

Before attempting to identify the bearing of the anterior lobe on the formation of the skeleton, it is essential that the principle or principles of each lobe should be known.

The Anterior Lobe. Evans in his Harvey Lecture (1923-24) comprehensibly reviewed the functions of this lobe. He established with certainty the presence of growth stimulating and ovulation inhibiting hormones in the pars anterior. Bugbee, Simond and Grimes (1931) in their review of the literature find that there are eleven different principles claimed:

(1) Stimulation of growth during the active growth period.

(2) Stimulation of sexual development and ripening of ovarian follicles resulting in ovulation.

(3) Stimulation of lutein cells, causing cessation of ovulation and imprisonment of ova.

(4) The enhancement of sexual maturity.

(5) Stimulation of metabolism by increasing specific dynamic actions of foods.

(6) Stimulation of the thyroid gland.

(7) Lowering of the basal metabolism.

(8) Increasing the water intake and excretion.

(9) Excitation of lactation.

(10) The lowering of the non protein blood nitrogen.

(11) The initiating of bleeding that comes with menstruation.
Wiggers (1935) claims that to these the following should be added:

(12) Modification of the carbohydrate metabolism which is antagonistic to the action of insulin.

(13) A trophic influence on the adrenal cortex.

(14) A stimulation of growth of the pancreatic islets.

(15) The regulation of fat metabolism.

(16) A melanophoric hormone concerned in mammals with the pigmentation of pregnancy, and with the adaptation of the pupil to light.

He further goes ontto state that "available evidence indicates that five or six substances or groups of substances can be separated from the anterior lobe that may tentatively be regarded as specific hormones. They are (a) the growth hormone, (b) the gonadotropic hormone, (c) the thyrotropic hormone, (d) the adrenotropic hormone, (e) the lactogenic hormone and (f) a diabetogenic factor.

The Posterior Lobe. The origin of the hormone has not been settled, the opinion most generally accepted is that the hormone comes from the cells of the pars intermedia and the pars tuberalis, and stored in the pars nervosa from which it was originally extracted. The route by which the hormone leaves is still an open question; that is, whether it goes directly into the blood stream or goes through the third ventricle into the cerbro-spinal fluid. Cushing (1932) believes that the hormones need only reach the hypothalamic region in sufficient concentration to stimulate centers of the para-sympathetic system and thus various vegetative functions of the body are then controlled through them. He demonstrated this experimentally by injecting pituitrin intraventricularly into human subjects.

Ablation of the posterior lobe does not cause any marked

physiological changes, and from this it is concluded that there is no essential hormone present.

Injection of extracts made from the posterior lobe show marked physiological responses, chiefly on the contractile structures, producing

(1) A sharp and sustained rise of the arterial blood pressure. Repeated injections may cause an inversion of the blood pressure, depressor response.

(2) Diuretic, anti diuretic action.

(3) Stimulation of the uterus, intestines and bladder.

(4) Secretory accelerator effect on the mammary gland, and an inhibitory action on the salivary gland.

(5) In amphibians, a very pronounced action on the pigmentary system, a melanophore-expanding action.

(6) An increased metabolic rate and temporary hyperglycemia.

A. Secretion of the Anterior Lobe.

The gonad stimulating, growth promoting, and thyrotropic hormones, as well as those responsible for the normal functioning of the adrenals, the pancreas and probably the parathyroids, seem to be found in the anterior lobe.

The eosinophilic cells are thought to secrete the growth promoting hormone, because (1) acromegaly is apparently due to an excessive secretion of the growth-promoting hormone by the eosinophilic cells of an adenoma and (2) Smith and Smith (1923) found that from ox pituataries made up of eosinophils and reserve cells growth-promoting effects could be produced. They found that if tissue composed of basophils and reserve cells was used, no growth promoting hormone was produced.

Nelson (1930) found that the pituitary of the foetal pig had a marked differentiation of the eosinophils when the pig is 16-17 centimeters. In younger fetuses the chromophils were chiefly basophilic; implants of these pituitaries caused growth in hypophysectomized rats, but demonstrated no gonadotropic effect in immature mice.

The work of Smith and Dortzbach (1929) and Nelson (1930) suggests that the basophilic cells in the pig secrete the growth hormone. However in the woodchuck, rat, rabbit, dog, the basophils are usually associated with the gonadotropic hormone.

IV. Experimental Gigantism and Dwarfism.

The most important step in throwing light upon the function of an endocrine gland is the observation of the effects produced when the gland is removed. The pituitary body however, due to its position has presented a great many difficulties. Its proximity to the hypothalamus and tuber cinereum, and because it is surrounded by a rich venous sinus, have made the gland very difficult to remove without injuring the neighboring structures. According to Van Dyke (1936) "Many of the discordant results in the literature concern hypophysectomy in the dog, are due to complications arising from injuries of the hypothalamus."

A. Results of Partial and Total Hypophysectomy.

Paulesco (1906) employed the lateral or temporal approach in order to expose the gland. Upon the removal of the whole

gland in twenty-two dogs, twenty-one died within a few days, one lived 365 days but when autopsied, it was observed that the gland was not completely removed. He then removed the anterior lobes in three dogs, who in turn displayed no noticeable symtoms. The removal of the posterior lobe in five animals disclosed no noticeable symptoms with regard to body structure.

Cushing (1909) demonstrated that partial removal of the anterior lobe was not incompatible with life. The animals in some cases became fat, lost sexual power, genital organs atrophied, and the hair fell out. The obesity may have been due to hypothalamic injury, the mechanism by which this occurs is clearly understood.

Aschner (1912) used the buccal approach as used by Paulesco (1906) and Cushing (1909), and using young dogs, observed that the removal of the whole gland was not fatal. His animals remained small in stature, the milk dentition remained and was taken on rapidly, and the genitalia did not atrophy or develop. The epiphyseal cartilages persisted, and the epiphyses did not close. There was in general, a well marked delay in the ossification and in the growth of the bones.

Dott (1923) removed about two-thirds of the anterior lobe of the pituitary in dogs with the result that there was a marked retardation of growth. In seven experiments he found that the average diminution was about 57 per cent. There were histological changes in the epiphyseal cartilages and in the osteogenetic cells of advancing ossification corresponding

with the conditions of diminished and increased activity. He observed that, when the posterior lobe was removed, there was no effect on the growth or development of the animals.

A great deal of work has been done with rats, and various techniques by which the gland can be removed either partially or totally have been described in papers by Smith (1927, 1930), Thompson (1932), Anselmino and Peucharz (1934). The method most commonly used is that of Smith, whereby the anterior and posterior lobes are exposed by a parapharyngeal (ventral) approach and removed with a cannula and negative pressure. There is no rupture of the diaphragma sellae or apparent brain injury. The stalk and pars tuberalis remain intact, but a constant syndrome results from the operation.

Smith (1930) attempted to determine which of the glands was most concerned with dwarfism in piebald rats. He used the parapharyngeal approach, and his results were in harmony with those of other investigators. "The hypophysectomized rats exhibit an almost complete stoppage of skeletal growth." "The ablation of the posterior pituitary alone does not cause any slowing of development." The epiphyses remained open, and the epiphyseal cartilages shrunk, then their cells underwent degeneration. These findings conformed with the results of Aschner (1912) and Dott (1923). The fact that the epiphyses remain open after hypophysectomy is not an abnormal condition in rats, for these animals continue to grow through most of their life and the epiphyses normally remain open until late

in life, according to Dawson (1925). From Smith's work it is seen that there is a definite failure in the growth of the skeleton. Schour and Van Dyke (1932) also demonstrated that after hypophysectomy there is a definite retardation of growth in the incisor teeth, which normally grow continuously. The removal of the gland also causes a delay in the eruption of the molars, and histologic changes in both the molars and incisors.

According to Smith (1927) by using a temporal approach, and then injecting chromic acid, the posterior and anterior lobes were nearly completely destroyed. There was a definite dwarfism produced, and the rats demonstrated the same results as obtained by the use of the parapharyngeal approach, except that with the chromic acid injections, a definite obesity was produced "due to an injury of the hypothalamus."

It has been observed that hypophysectomy, partial of complete, in young and mature animals inhibited growth. Therefore, using an apriori argument, the administration of the hypophysis (part or whole) or of extracts made therefrom would cause a resumption of growth in animals that were abnormally small because of previous hypophysectomy.

B. The Effects of Replacement Therapy.

According to Van Dyke (1936), the term growth depends on the manner in which the investigator uses it. Some investigators attribute growth to an increase in body weight. In experimental gigantism, growth is a more prominent change than that of body dimensions or of some of its parts. "A truer growth-promoting effect is obtained by the administration of the

anterior pituitary to animals hypophysectomized when young; in such animals the body weight and body size are strikingly increased by the treatment." Feeding, transplantation, implantation, and the injection of extracts are the methods most commonly used. Since the condition known as acromegaly was associated with the hyperfunction of the pituitary body, a number of experimenters have attempted to bring about this condition by feeding the whole gland on the anterior lobes or extracts of the gland as an anterior lobe in order to bring about this condition. The animals used were worms, amphibia and mammals.

Schaefer (1909) fed four young rats small amounts of dried anterior lobe mixed with bread and milk. The controls were fed testicle or ovary with bread and milk. The amounts consumed were accurately determined. This experiment was carried on for a period of three months. At the beginning of the experiment the test and control animals each weighed 44.25 grams. The first six weeks of the work demonstrated no difference in the growth of the two groups. During the last six weeks however, the test animals grew very rapidly and the final result disclosed that the test animals weighed 150 grams, the controls 131 grams.

Schaefer's work was merely a stepping stone for other investigators using different animals. Smith (1918) fed fresh anterior lobes of the ox pituitary to normal and hypophysectomized tadpoles (Rana Boylei).. The normal animals which were

fed anterior lobes grew much more rapidly than did the controls. The hypophysectomized tadpoles grew as rapidly as the normal ones up to the mid-larval stage, then there was a drop in the growth rate. These animals, when fed anterior lobes, displayed a marked increase in growth. Metamorphosis did not occur, and the tadpoles grew for a longer period and to a larger size than normal tadpoles without pituitary feeding.

Ulenhuth (1921-1923) using salamanders (Amblystoma tigrinum, Amblystoma opacum) after metamorphosis, fed the anterior lobe of ox hypophysis to one group, liver to another and earthworms to a third groups. He had controls for each group. The length, sex and weight were carefully recorded at the beginning of the experiment. The hypophysis fed salamanders grew to very great lengths, much greater than those fed liver, or earthworms. He believes that the anterior lobe contains a substance which maintains growth for a longer period and a larger size than liver or earthworm fed animals. The difference in the effects produced is one of quality and not of quantity, and therefore, the anterior lobe substance produces gigantism in certain salamanders.

Downs (1927) using puppies ranging from four to six weeks of age, the genetic makeup of which was not known. He divided the dogs into two groups. Group 1 consisted of five litters of four dogs each. He fed anterior lobes to the first, posterior extract to the second, and whole gland extract to the third three times a day and used the fourth dog as a con-

trol animal. His results as to increase in skeletal structure were negative. There were no variations in the size, shape, number and arrangement of the cells in the hypophyses, thyroids and parathyroids.

Robertson and his coworkers (1916, 1919, 1920, 1923) claimed to have prepared a substance which was called "Tethelin" from the anterior lobe of the ox pituitary, and which, if fed to white mice, stimulated growth. Drummond and Cannan (1922) found that "Tethelin" had no growth-promoting properties and thereby disproved Robertson's work. Evans and Long (1922) could get no growth increase in rats by feeding anterior lobe preparations. Smith (1927) fed two fresh anterior lobes of ox pituitaries to each rat that he had hypophysectomized, and observed no increase in the growth, either in the size or in the body weights of the animals. These men all arrived at the same conclusion, that feeding the gland either wholly or in parts did not stimulate growth.

V <u>The Growth Hormone.</u>

A. Effect of Administration of the Growth-promoting Hormone.

Evans and Long (1921) demonstrated that giant rats could be produced experimentally by the injection of saline extracts of the anterior lobe. They dissected out the anterior lobes, immersed them in a 30 per cent solution of alcohol, then rinsed thoroughly with Locke's solution titrated with a small amount os sand, and then centrifuged for about a half hour, aseptic precautions were closely watched. The supernatant fluid that

resulted from the centrifuging was injected into the peritoneal cavity in amounts that ranged from one-eighth cubic centimeters, according to the age of the rat. The animals were weighed at five day intervals.

This piece of work by Evans and Long (1921) stimulated the interest of a great many experimental biologists. Smith and Smith (1922) injected suspensions of the different parts of the hypophysis of the ox into hypophysectomized and normal tadpoles. The hypophysectomized tadpoles grew larger than the normal tadpoles, and the atrophic changes in the adrenal cortex, the epithelial bodies (parathyroids) and the thyroids were corrected. These effects were produced by suspensions of anterior lobe, especially suspensions prepared from the portion containing oxyphilic (eosinophilic) and reserve cells.

Smith (1927) demonstrated that an anterior pituitary extract could cause the resumption of growth in hypophysectomized rats. He (1930) injected suspensions of the anterior lobe and observed the resumption of growth in the rats that were dwarfed through hypophysectomy. There was a definite growth change in the skeleton, much greater than occurred when normal animals were injected with the growth hormone. The atrophic thyroid, adrenals, gonads and pancreas took on a normal functioning appearance after the replacement therapy was started.

Evans and Long (1924) observed that unless large doses of growth promoting extract were used, the differences between

normal and injected growing rats showed no striking results until after they had reached the age of 75 to 100 days. After this time normal rats grew more slowly, and injected rats grew much more rapidly. At 200-400 days of age, the injected rats were twice as heavy as the normal ones. The skeleton and the viscera were markedly enlarged.

Putman, Benedict and Teel (1929) used two pedigreed thoroughbred English bulldogs, which were female littermates four weeks old. The dogs were kept under observation for three weeks before the actual experiment was started and it was noticed that they were normal puppies. The dosage used started at 10 cubic centimeters daily, and this was increased to seventy-five cubic centimeters. The test animal received doses of the extract for fourteen months. At death the weight of the test animal was 44 kilograms and that of the control 23.3 kilograms. The skull of the treated animal was much thicker than that of the control, and the teeth were longer and more widely spread. The long bones were enlarged, on their prominences were many loose osteophytes best seen in the tibia. The patella also displayed osteophytic changes. The 'acral parts' were definitely enlarged and the mandible was elongated. The thyroid showed abnormally dense cellular structure and no colloid in the alveoli, which were collapsed and irregular in shape. There was a definite hypertrophy of the liver, the adrenals, the heart, the kidneys and the genitalia.

Downs (1927) used six litters of three dogs each. One

dog received three minims of antuitrin (Parke Davis & Co.), the second dog three minims of pituitrin (Parke Davis & Co.), and the third dog sterile water, table 2.

TABLE 1

April 7-May 1 Measurements of Animals Used in Experiment

Animal	Greatest diameter of head in cms.	Diameter of snout cms.	Chest, cms.	Olecranon tottip of toe, cms.
Experimental	38	22	52	26
Control	34	22	49	25

December 8

Animal	Greatest diameter of head in cms.	Diameter of snout cms.	Chest, cms.	Olecranon to tip of toe in cms.	Occiput to Coccyx. cms.
Experimental	49	26	64	30	73
Control	41	24	66	27	64

After Putnam, Benedict and Teel (1929)

TABLE 2

Animal	L-component		H-component	Time killed
#10 (anterior)	880	to	1000	56 days
#11 (control)	820	"	"	"
#12 (posterior)	875	"	"	"
#13 (anterior)	NO EXAMINATION.			
#14 (control)				
#15 (posterior)				
#16 (anterior)	880	"	"	65 days
#18 (control)	870	"	"	"
#17 (posterior)	820	"	"	"
#4 (anterior)	890	"	"	83 days
#6 (control)	870	"	"	"
#5 (posterior)	820	"	"	"
#7 (anterior)	895	"	"	83 days
#9 (control)	870	"	"	"
#8 (posterior)	825	"	"	"
#1 (anterior)	895	"	"	99 days
#3 (control)	875	"	"	"
#2 (posterior)	825	"	"	"

After Downs (1927)

Short bone component was taken from the crest on the medial border of the scapula to posterior superior iliac spine when the animal lay on its back with the legs extended at rt. angles to the long axis of the vertebral column.

Long bone component was measured from the sole of the forefoot pad to the eminence on the medial border of the scapula, the animal in the same position.

$L = \frac{\text{Short bone}}{\text{Long bone}}$ $H = 1000$

Handelsman and Gordon (1930) workin with rats demonstrated that the growth hormone extract as prepared by Evans and Simpson had bone growth stimulating qualities. They used 175 rats ranging in weight from 30 grams to 350 grams. In animals ranging from 30 to 100 grams, the potency of the extract could not be detected. In rats ranging from 175 to 225 grams, they observed an average increase of 2.1 grams in weight per day after intraperitoneal injections of one cubic centimeter daily for a twelve day period.

The authors fed the rats a standard diet plus madder which vitally stained the bones for two weeks before starting the injections. In young animals ranging from 30 to 100 grams in weight, the madder was spread diffusely over the surfaces of the bones, due to the great periosteal activity that was present. In animals ranging from 175 to 300 grams the periosteal activity was limited to definite zones which are more or less constant for different age groups.

The skull bones took on more madder than did the other bones, and since these were easily obtained, the workers examined the premaxilla, the body of the maxilla, the entire zygomatic arch (including the zygomatic process of the maxillary, jugal and squamosal bones), the alveolar process, the palatine bone and the process palatinous of the maxilla. After injections for twelve days, the bones presented an intensification of the normal ossification zones, which was due to the over stimulation of the normal periosteal bone growth. The investigators also

found that "the response of the osseous tissue to the growth hormone is roughly parallel with the growth incremants of the entire animal."

Lucke and Huckel (1933) observed proliferative changes in the joint cartilages as well as proliferative and retrogressive changes in the epiphyseal cartilage, all of which they attribute to the administration of a growth promoting extract. They conclude that the alterations resembled those accompanying the specific arthritis of early human acromegaly.

Johnson and Sayles (1929) got only a slight increase in the bone growth of rats. Their reason is: "apparently an excess of anterior growth hormone is not strikingly effective in the production of the long bones before the 150th day, but tends to produce a general increase in growth. Excessive skeletal growth appears to be made possible by a delay in the complete ossification of the long bones in the anterior pituitary injected rats. The ossification occurs at about 150 days in the normal female rat and at about 200 days in the normal male." Johnson and Hill (1930) using mice, injected one half group with anterior pituitary growth hormone extract, the other half, controls were injected with a .9 per cent saline solution. The maximum disage was .5 cc. daily. After 130 days of treatment, the animals were killed and the body measured from the tip of the nose to the base of the tail, and from the base of the tail to the full tip, and from the anterior edge of the acetabulum of the right hind leg to the edge of the longest toe. The increase in growth was more marked in females. (Table 3)

TABLE 3

	Body	Tail	Hind Leg
Average length of experimental females in mm.	97.2	78.7	31.9
Average greater length as compared with controls in mm.	3.8	0.0	0.4
No. of females longer than controls	17	12	11
No. of females same length as controls	0	0	2
Nor. of females shorter than controls.	4	7	8
D/E	4.2	1.7	1.0
Average length of all experimental males in mm.	92.8	78.4	31.7
Average greater length as compared with controls in mm.	3.5	0.8	0.9
No. of experimental males longer than controls	15	10	12
No. of experimental males same length as controls	0	2	2
No. of experimental males shorter than controls	3	6	4
D/E	4.3	1.4	2.8

Tests		Controls
Females	21	11
Males	14	14

Apte Johnson and Hill (1930)

Reichart (1928) hypophysectomized a female puppy six weeks of age. After six months had passed, the animal weighed 4.2 kilograms, was infantile in stature and development, and the permanent dentition failed to erupt. The control, however, attained adult size and weighed 9.3 kilograms. The test animal was injected subcutaneously with fresh whole rabbit hypophysis for a two month period, showing very little skeletal growth. However, after one month of injection, the permanent dentition appeared. Skeletal growth was progressing slowly due to elapse of six months before injections were started. In (1929), Reichart again totally hypophysectomized an eight week old female puppy which was kept under observation for four weeks after the operation. During this time the tibia and femur increased 6 mm. in length, the skull 12 mm. in thickness, and 32 grams in weight. The control during this time increased 33 mm in the length of the leg, the skull 12 mm in length and 88 grams in weight. After giving the test animal 25 cc. daily of Evans' growth hormone extract intraperitoneally for ten weeks, there was a marked increase in skeletal growth and weight. The femur and tibia increased 70 mm in length, the skull 10 mm in length and the weight 164 grams. The control gained during this period 222 grams, the femur and tibia 53 mm, and the skull 10 mm. Rubenstein and Kolodner (1934), using 74 adult rats found that 2 cc. of alkaline aqueous extract of anterior beef lobes daily injected into these animals had a definite effect, the body length increased, and the tail length increased, therefore there was a total increase

in the length of the animal. However, they observed that the females responded much better than did the males. If the administration of the growth promoting extract is delayed and the rats are growing slowly (i.e. weight and size), and if the period of administration is about three weeks, female rats gain more weight and a relatively greater skeletal growth than do the males.

Evans, Meyer, Simpson, and Reichart (1933), using shepherd dogs of known genetic constitution, injected them for over a period of a year with their growth hormone prepared by the acetic acid quinine method. They hoped to produce acromegaly in these animals by stimulating the body weight and skeletal overgrowth in the animals. However, roentgenograms of the skull, femur, and tibia, taken at the standard distance of six feet, disclosed that the largest tibia and femur of the test animals exceeded those of the controls by only 2.4 cms., the largest skull exceeded those of the controls by only 1.7 cms. There was no abnormality in the closure of the epiphyses. However, there was a slight acromegaly and gigantism produced, which did not correspond to the results obtained by Putnam, Benedict and Teel (1930). Using pure bred dachshunds which are achondroplastic dwarfs, they attempted to increase the size of the body (skeleton and weight). The bones were found to be larger, the standing height was slightly increased, a marked gigantism was produced. The vertebrae and skull displayed a definite increase in length and breadth. (Table 4)

TABLE 4

	HY24 Control Male					HY25 Injected Male				
Date (age) Weeks	Dose cc.	Weight kgs.	Femur & Tibia cms.	10 Vertebrae cms.	Skull cms.	Weight kgs.	Femur of Tibia cms.	10 Vert.		Skull cms.
4/9/31 (6)	1-1.5	1.98	7.9		9.5	1.86	7.6			9.4
4/30/31 (9)	2.5	3.55	10.2		11.3	3.75	9.6			11.3
5/14/31 (11)	2.5-5.0	3.70	10.6		12	4.10	10.7			12.3
6/4/31 (14)	7-10	5.16	12.4		13.1	6.90	12.5			14.0
7/6/31 (19)	10	6.60	13.5		14.4	9.4	14.6			16.3
8/12/31 (24)	10	7.62	14.9	18.6	15.7	12.88	16.7	20.7		18.0
9/10/31 (28)	10	8.58	15.7	19.5	15.7	16.22	17.4	22.1		19.4
10/8/31 (32)	10 stopped 12/29/31	9.36	16.2	21.2	16.2	18.42	18.6	24.16		20.4
12/1/31 (40)		10.92	16.9	22.1	16.2	17.10	19.6	25.0		20.8
1/14/32 (46)		11.19	17.1	22.1	16.2	16.28	20.1	25.3		21.0
3/8/32 (54)		10.92	17.1	22.7	16.8	16.40	20.1	25.5		21.2
4/18/32 (60)		12.34	17.1	22.7	16.8	16.62	20.1	25.7		21.2

TABLE 4 (continued)

	HY27 Control Female					HY26 Injected Female			
Da (age)	Dose	Weight	Femur of tibia	10 Vertebrae	Skull	Weight	Femur of tibia	10 vert.	Skull
Weeks	cc.	kgs.	cms.	cms.	cms.	kgs.	cms.	cms.	cms.
4/9/31 (6)	1-1.5	1.64	7.8		8.8	1.54	7.9		8.4
4/30/31 (9)	2.5	3.10	9.6		10.5	3.44	9.9		10.4
5/14/31 (11)	2.5-5	2.80	10.7		11.0	3.96	11.0		11.2
6/4/31 (14)	7-10	4.00	12.4		12.0	5.86	13.0		12.5
7/6/31 (19)	10	5.00	13.9		13.1	6.70	14.8		14.3
8/12/31 (24)	10	5.52	15.2	17.3	14.0	9.28	16.9	20.2	15.7
9/10/31 (28)	10	6.34	15.8	18.5	14.2	10.88	17.9	22.1	17.3
10/8/31 (32)	10	6.74	16.3	19.0	14.4	12.62	19.1	22.8	17.5
12/1/31 (40)	10	7.12	16.3	19.1	14.7	12.60	19.9	24.2	17.8
1/14/32 (46)	10	7.46	16.5	19.4	14.8	14.54	20.1	25.1	18.0
3/8/32 (54)	10	8.12	16.5	19.4	14.8	15.46	20.2	25.5	18.2
4/18/32 (64)	10	10.0	16.5	19.4	14.8	16.80	20.2	25.5	18.2

On 1/14/32, the proximal epeiphyses closed in both animals, but the distal ends were open.
On 3/8/32, the epiphyses of the long bones of both animals were closed.

B. The Preparation and Properties of the Hormone.

There have been many attempts to prepare a purified growth hormone. The procedure is difficult because of the complexity of its structure. Evans (1929) attributes the difficulty of the purification of the hormone to:

1. It is highly complex and behaves like a protein.
2. It is very labile.
3. A chemical test is not non-existent, and a short reliable biological assay method is not available.

Various methods were devised to prepare the hormone, after Evans and Long (1921) came out with their revealing results. Putnam, Benedict and Teel (1928), Hewitt (1929), Van Dyke and Wallen-Lawrence (1930), and Collip, Selye and Thomson (1933) have all prepared extracts which have been demonstrated to be potent.

At the beginning, it was a question of separating the growth stimulating hormone from the sex hormone, but now it is a problem of getting the hormones present in the anterior lobe into a purified form.

The usual procedure for preparing the growth hormone is to extract the hormone from fresh bovine anterior pituitaries by treating the glandular tissue with either $NaOH$, NH_4OH, $Ba(OH)_2$, or $Ca(OH)_2$. Subsequent purification of the hormone contained in the alkaline extract has been attempted by a number of methods. Teel (1929) used sodium sulfate to salt out the extract. Collip and his coworkers (1933) used $Ca_3(PO_4)_2$ which, under proper conditions adsorbed the hormone, and the hormone could be easily separated from the adsorbent. By adjusting the hydrogen ion

concentration to a pH of 7.5-8, the hormone was separated. They however, claimed to have obtained a hormone that was free from gonad and thyroid stimulating effects.

Evans, Meyer and Simpson (1933) in their publication describe a great many methods for the purification of the hormone. They used phosphotungatic acid, flavianic acid, trichloroacetic acid, iso-electric precipitation, and a few more. Their methods were very complex as well as the physical-chemical changes involved.

Very little is known about the properties of these extracts. They appear to be heat labile, they may be able to stay fifteen minutes at a temperature of about 60°C, others may be inactivated at lower temperatures.

The various extracts have different potencies. A dose of .09 cc. of the extract prepared by Van Dyke and Wallen-Lawrence (1930), for a 250 gram female per day given for a period of three days produced a total increase in growth approximating 3 per cent. The total dose 0.27 cc. contained about 2.7 mg. of total solids in part made up of protein. Most of the solids were salts. Collip, Selye and Thomson (1933) used a dose of one cubic centimeter twice a day and observed marked growth in totally hypophysectomized rats. Evans, Meyer and Simpson (1933) tested their extracts on rats and dogs and found them to be very potent.

C-MECHANISM OF THE ACTION OF THE GROWTH HORMONE.

The manner in which the growth hormone acts is still unknown. Evans (1935) believes that the hormone either acts directly on the tissues or stimulates other endocrines to over functioning and these glands in turn give off their secretions which act directly on the tissues. The latter opinion seems to be a matter of speculation on the part of Evans, but he does not offer any explanation. Therefore the method of action is still unknown.

Allen (1916 and 1928) observed the growth of hypophysectomized and thyroidectomized tadpoles when anterior lobe extracts were injected into the animals. The thyroids of the hypophysectomized animals were the only glands that were observed to have undergone any change. With the replacement treatment, using the growth hormone, there was an increase in growth and also an improvement in the subnormal structure of the thyroid. The thyroidectomized tadpoles did not respond to the treatment at all. This seems to imply that the growth hormone does act on the thyroid but does take over thyroid activity when the gland is not present.

Teel and Watkins (1929) analyzed the blood of fasting dogs before they were injected with growth hormone extracts and after injections were given. They found slight changes in the blood contents of the injected dogs. There was a slight drop in the serum calcium, and also slight changes in the inorganic phosphorous. However, the total analysis of the blood of two dogs, which had been made definitely acromegalic by the growth hormone

injections, did not demonstrate any definite changes in the blood composition, as compared with the blood composition before injections were undertaken.

They believe, moreover, that the growth hormone has an immediate effect on the non protein constituents for they state, "It seems reasonable that this effect might be, at least in part, one of mobilization from the blood for the purpose of building up new protoplasm." Teel and Cushing (1930) also observed slight changes in the serum calcium and inorganic phosphorous after injections of the growth hormone. The nitrogen drop observed in the blood was found not to have been excreted and the authors believed that it was stored up in the tissues. The experimenters tried to check up on the nitrogen, calcium and phosphorous after the animals were fed a modification of Corrigill's synthetic diet. Despite the constant diet, urine analyses demonstrated no retention of nitrogen and there was no trace of phosphorous observed, but the calcium was increased.

The manner by which this hormone activates the skeleton is still very doubtful. How does the calcium get into the blood stream, which in turn affects the bones? If there was an observed stimulation of the parathyroids, there would be some clue to the manner by which the hormone acts, but Putnam, Teel and Benedict (1929), Down (1927), Smith (1927) (1930), Evans et al. (1933), have observed no trace of this action as having taken place. Collip (1934) however, has observed that with hypophysectomy in the cat, the parathyroids were enlarged. This was one of the only pieces of work that gave some evidence that

the parathyroids were connected with the pituitary. He further states that in the hypophysectomized rat, the calcium in the blood was observed to have dropped. After injection with the growth hormone, there was a restoration of the calcium balance in the rat that had been dwarfed in skeletal size.

However, until the chemistry of the gland is known, and until the hormones are prepared in purer form, it must be taken for granted that the growth hormone does influence skeletal formation and growth.

VI SUMMARY

1. The hypophysis cerebri or pituitary body consists of two lobes, the anterior and the posterior. The anterior lobe is glandular in structure, the posterior lobe is composed of nervous and connective tissue.

2. The anterior lobe is believed to elaborate six known hormones. The hormones are secretions of the alpha (eosinophils, oxyphils) or beta (basophils, acidophils) cells.

3. Hypophysectomy total or partial has been demonstrated as bringing about a definite stopping of the growth of the body as well as the skeleton, producing dwarfism. Overstimulation of the gland brings about the condition of acromegaly and gigantism.

4. The anterior lobe has been definitely associated with gigantism, and acromegaly, due to a hyperfunction of this lobe, or dwarfism, which is attributed to a hypofunction of this portion of the gland.

5. Feeding experiments did not cause a hyperfunctioning of the anterior lobe, and therefore no gigantism.

6. The development of gigantic rats in 1921 by Evans and Long, after intraperitoneal injections of alkaline preparations of the anterior lobes of beef hypophyses demonstrated that there must be a hormone which controls growth, separate from the hormone controlling the gonads.

7. The definite production of acromegaly in dogs by injections of the growth hormone by Putnam, Benedict and Teel (1929), and the results obtained by them aided further in the development of the prescence of the growth hormone, which brought about this condition.

8. The growth hormone brings about a definite growth of bone, as proved by Handlesman and Gordon (1930). Lucke and Huckel (1933), Downs (1927), Smith (1927) (1930), Evans et al. (1933).

9. The growth hormone controlling skeletal as well as body growth, has been purified (rather crudely) and definitely separated from the other hormones.

10. The bio chemical and mechanical action of the hormone which produces gigantism or dwarfism is not definitely known.

11. With the knowledge that is now at hand, it is possible that the hormone either stimulates the parathyroids or that the hormone acts directly on the body tissues in producing either gigantism or dwarfism.

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